Enhanced degradation of micropollutants by zero-valent aluminum activated persulfate: assessment of toxicity and genotoxic activity

T. Olmez-Hanci, I. Arslan-Alaton, M. Doğan, S. Khoei, H. Fakhri
and G. Korkmaz

ABSTRACT

Advanced oxidation of the aqueous Triton™ X-45 (TX-45), iopamidol (IOPA), ciprofloxacin (CIP) and bisphenol A (BPA) solutions via activation of persulfate (PS) with zero-valent aluminum (ZVA) was investigated. The study aimed at assessing the effectiveness of the PS/ZVA process in terms of target micropollutants (MPs) and toxicity abatements in raw surface water (RSW) and distilled water (DW). TX-45, CIP and BPA were completely degraded after 90-minute, 120-minute and 40-minute treatment, respectively, with PS/ZVA in DW, whereas 95% IOPA removal was achieved after 120-minute (MPs = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM for CIP and BPA; PS = 0.50 mM for TX-45 and IOPA; pH = 3). TX-45 (59%), IOPA (29%), CIP (73%) and BPA (46%) removal efficiencies decreased after 120-minute PS/ZVA treatment in RSW. In DW, *Vibrio fischeri* toxicities of original (untreated) MPs were found as: CIP (51%) > BPA (40%) > TX-45 (15%) > IOPA (1%), and as BPA (100%) > CIP (66%) > IOPA (62%) > TX-45 (35%) in RSW. Acute toxicities of MPs and their degradation products fluctuated during PS/ZVA treatment both in DW and RSW samples and resulted in different relative inhibition values after 120-minute. The original and PS/ZVA-treated TX-45, IOPA and BPA in DW exhibited neither cytotoxic nor genotoxic effects, whereas CIP oxidation ended up in degradation products with genotoxic effects.

Key words | acute toxicity and genotoxicity, bisphenol A, ciprofloxacin, iodinated X-ray contrast media, nanoscale zero-valent aluminum, non-ionic surfactants

T. Olmez-Hanci (corresponding author)
I. Arslan-Alaton
M. Doğan
S. Khoei
H. Fakhri
G. Korkmaz
School of Civil Engineering, Department of
Environmental Engineering,
Istanbul Technical University,
34469 Maslak Istanbul,
Turkey
E-mail: *tolmez@itu.edu.tr*

INTRODUCTION

In the past decades, the presence of micropollutants (MPs) in water sources has raised considerable interest due to their potential harmful environmental and health impacts. The MPs group includes a wide variety of compounds such as pharmaceuticals, personal care products, hormones, industrial additives and household chemicals (Luo *et al.* 2014). The main sources of MPs are wastewater treatment plants for domestic sewage, wastewater from hospital effluents, chemical manufacturing plants, livestock and agriculture (Luo *et al.* 2014; Pasquini *et al.* 2014). Major consequences of MPs are feminization of higher organisms, microbiological resistance and accumulation in soil, plants and animals, short-term and long-term toxicity, endocrine-disrupting effects, and antibiotic resistance of microorganisms (Fent *et al.* 2006). Recent studies demonstrated that

conventional water/wastewater treatment plants are inadequate to effectively remove MPs, due to their complex molecular structure, chemical stability and low biodegradability (Gavrilescu *et al.* 2015). On the other hand, advanced oxidation processes (AOPs), characterized by the generation of highly reactive, non-selective free radicals such as sulfate ($SO_4^{\bullet-}$, $E^0 = 2.6$ V) and hydroxyl ($HO^{\bullet}$, $E^0 = 2.7$ V), offer a promising alternative to conventional treatment for the removal of MPs in water or wastewater. In the past years, persulfate (PS, $S_2O_8^{2-}$) has attracted increasing attention in AOP applications since it is more stable than hydrogen peroxide (HP) and has a relatively high redox potential ($E^0 = 2.01$ V). Upon thermal, chemical or photochemical activation, it is possible to generate $SO_4^{\bullet-}$ from PS (Antoniou *et al.* 2010).

$SO_4^{\cdot-}$ generated in activated PS processes is more selective than the $HO^{\cdot}$ for the oxidation of compounds with carbon-carbon double bonds and benzene rings (Anipsitakis & Dionysiou 2004; Huang *et al.* 2005). The activation of PS with nanoscale zero-valent iron and aluminum (ZVA) has exhibited a great potential in the oxidative treatment of refractory contaminants (Bokare & Choi 2009; Girit *et al.* 2015; Arslan-Alaton *et al.* 2017a, 2017b). Under acidic pH (pH < 4) and in the presence of dissolved oxygen (O_2), the $ZVA/H^+/O_2$ treatment system involves two major processes:

(i) corrosive dissolution of Al^{3+} and simultaneous reduction of O_2 to hydroperoxyl radical(s), $HO_2^{\cdot}$, that leads the formation of hydrogen peroxide (H_2O_2, HP); and

(ii) generation of $HO^{\cdot}$ by an electron transfer mechanism from ZVA to HP (Bokare & Choi 2009).

$$Al^0 \rightarrow Al^{3+} + 3e^- \tag{1}$$

$$O_2 + H^+ + e^- \rightarrow HO_2^{\cdot} \tag{2}$$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \tag{3}$$

$$Al^0 + 3H_2O_2 \rightarrow Al^{3+} + 3HO^{\cdot} + 3OH^- \tag{4}$$

The oxidation capacity of the $ZVA/H^+/O_2$ system relies on the formation of $HO^{\cdot}$, which possesses strong oxidizing capability towards a variety of organic compounds. For example, the removal of aqueous acetaminophen [N-(4-hydroxyphenyl) ethanamide, ACTM] using the $ZVA/H^+/air-O_2$ system was investigated by Zhang *et al.* (2012). More than 99% of 2 mg/L aqueous ACTM was removed in 16 h, at pH 1.5 and $25 \pm 1\,°C$ by the $ZVA/H^+/air-O_2$ treatment system and higher ZVA concentrations enhanced the removal. Bokare & Choi (2009) examined 4-chlorophenol (4-CP) as the model pollutant to explore the potential of $ZVA/H^+/air-O_2$ treatment. Nearly complete (>95%) 4-CP degradation was achieved in 10 h under air-O_2 equilibrated conditions (ZVA = 1 g/L; 4-CP = 100 μM; initial pH = 2.5). The addition of oxidants (e.g. HP or PS) is expected to substantially improve the degradation rates of organic pollutants via ZVA-mediated treatment processes (Girit *et al.* 2015; Arslan-Alaton *et al.* 2017a, 2017b). It has been reported that in the presence of ZVA, PS could be activated under acidic conditions through direct electron transfer from the ZVA surface to PS to generate $SO_4^{\cdot-}$ (Arslan-Alaton *et al.* 2017a, 2017b):

$$2Al^0 + S_2O_8^{2-} + 6H^+ + 1.5O_2 \rightarrow 2Al^{3+} + 2SO_4^{\cdot-} + 3H_2O \tag{5}$$
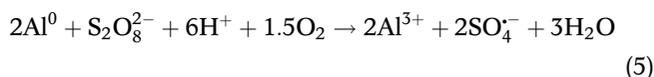
In a recent study, Cheng *et al.* (2015) examined the performance of acid-washed ZVA (AW-ZVA) in the presence of HP to remove phenol. The effect of HP addition (0–8 mM) on phenol removals by AW-ZVA was also investigated in this study. Increasing the initially added HP concentration from 1 mM to 2 mM increased the phenol removal efficiency; however, further increase did not improve phenol degradation.

During the application of AOPs, the main concern is associated with the formation of various degradation products that can potentially be more toxic, estrogenic, teratogenic, mutagenic, carcinogenic, genotoxic and/or persistent than the original compound/pollutant (Rizzo 2011; Garcia-Käufer *et al.* 2012). Thus, information provided by the target pollutant measurements alone is not sufficient to assess the effectiveness of the AOPs and their risks associated with the degradation products. In addition, the natural water matrices may change the degradation products formed during the application of AOPs, which in turn may lead to different levels of biological activity depending on the variability in the water quality characteristics. Thus bioanalytical tools are necessary to examine the impact of individual MPs and their degradation products but also the effects exerted by water/wastewater as a whole (Papa *et al.* 2016). Among the variety of bio analytical tools currently studied, a set of bioassays must be conducted for ecotoxicological assessment. As recommended by Escher *et al.* (2014), the 'ideal' battery test should consist of indicator bioassays that are able to cover a wide range of cellular toxicity pathways and take into account each mode of action: the non-specific (baseline), the specific (e.g. estrogenicity) and the reactive (e.g. genotoxicity) toxicity.

In the present work, a PS/ZVA treatment process for the removal of MPs in distilled water (DW) and raw surface water (RSW) spiked with MPs was investigated. Four extensively used chemicals that enter the aquatic environment through different discharge routes and ultimately form MPs were selected:

(i) a diphenyl methane plasticizer, bisphenol A (BPA)

(ii) an octylphenol ethoxylate-based non-ionic surfactant, Triton™ X-45 (TX-45)

(iii) an iodinated aromatic X-ray contrast medium, iopamidol (IOPA)

(iv) a fluoroquinolone-type antibiotic, ciprofloxacin (CIP).

Treatment performance was assessed in terms of target pollutant removals. The ecotoxicological effects were measured on two target organisms: the photobacterium *Vibrio fischeri* (*V. fischeri*) and the microalga *Pseudokirchneriella subcapitata* (*P. subcapitata*) in order to focus on taxa playing different roles in the trophic web (primary decomposer and producer, respectively). The genotoxicity of untreated and PS/ZVA-treated effluents was evaluated with the UMU-Chromo test, which has been developed and standardized to detect the genotoxicity of native, non-concentrated aqueous samples (ISO 13829 2000), and has been proposed as a screening test for monitoring the genotoxicity of individual pollutants as well as surface and wastewater samples (Hamer et al. 2000; Žegura et al. 2009).

MATERIALS AND METHODS

Materials

Commercial-grade ZVA nanoparticles (Brunauer–Emmett–Teller surface area: 10–20 m^2/g; particle size: 100 nm; purity: >99.9%) were purchased from US Research Nanomaterials, Inc. (Houston, USA). TX-45, with an average ethoxylate chain length of approximately five, $((C_2H_4O)_nC_{14}H_{22}O$; CAS Nr. 9002-93-1; purity: 98%) was purchased from Merck (Germany). IOPA (777 g/mol; $C_{17}H_{22}I_3N_3O_8$; CAS Nr. 60166-93-0) is a non-ionic radiographic contrast agent commercially available as 'Pamiray 300/370' injectable solution. IOPA was purchased from BIEM Pharmaceutical Co. A.S. (Ankara, Turkey). BPA (228 g/mol; $C_{15}H_{16}O_2$; CAS Nr. 80-05-7; purity: 99.9%) and CIP (331 g/mol; $C_{17}H_{18}FN_3O_3$; CAS Nr. 85721-33-1; purity ≥98%) as well as potassium persulfate (PS; 270 g/mol; $K_2S_2O_8$; CAS Nr. 7727-21-1; >99.5%) were purchased from Sigma-Aldrich (USA). Chromatographic-grade acetonitrile and methanol (MeOH) were obtained from Merck (Germany). Aqueous MP solutions were prepared in DW (Arium 61316RO, Sartorius AG, Germany). The RSW sample was taken from the influent of a local water treatment plant located in Istanbul, Turkey, treating lake water (total organic carbon = 7.6 mg/L; dissolved organic carbon (DOC) = 7.2 mg/L; suspended solids = 12 mg/L; color = 48 Pt-Co units; alkalinity = 115 mg $CaCO_3$/L; hardness = 110 mg $CaCO_3$/L; PO_4^{3-} = 0.17 mg/L; SO_4^{2-} = 15.5 mg/L; NO_3^- = 2.2 mg/L; Cl^- = 21 mg/L; pH = 7.9).

Experimental procedures

Aqueous MP solutions were treated in a 500 mL-capacity borosilicate glass reactor at room temperature (25 ± 2 °C) at fixed initial MP concentrations of 2 mg/L (BPA = 8.8 µM; TX-45 = 4.7 µM; IOPA = 2.6 µM; CIP = 6.0 µM) based on the calculated detection limits for both high performance liquid chromatography and bioassays and enabled direct analytical assessment of individual samples without further concentration. PS oxidation (in the absence of ZVA) of MPs was examined at pH 3 for 120 min. The more conventional ZVA/H^+/O_2 (in the absence of PS) treatment was explored at pH 3 with 1 g/L ZVA as a control experiment. PS/ZVA treatment experiments were conducted with 2 mg/L MPs aqueous solutions in the presence of 1 g/L ZVA at pH 3 for 120 minutes. The selection of the studied working conditions was based on experiments that have been reported recently (Arslan-Alaton et al. 2017a, 2017b). The experimental procedure of a typical run was described elsewhere (Girit et al. 2015). Samples were taken at regular time intervals from the reactor and filtered through 0.22 µm Millipore membranes (Millipore Corp., USA) to separate ZVA from the reaction solution. All experiments were conducted in duplicate and average values were taken when presenting the results.

Analytical procedures and instrumental analyses

An Agilent 1100 system (Agilent Technologies, USA) with a diode array detector was employed for the measurement of MPs. The chromatographic conditions used for BPA, TX-45 and IOPA have been described elsewhere (Girit et al. 2015; Arslan-Alaton et al. 2017a, 2017b). For CIP measurement, a C18 Novapack column (3.9 mm × 150 mm; 5 µm particle size; Waters, USA) was employed as a stationary phase, while the mobile phase was a mixture of 0.1% CH_2O_2/MeOH-H_2O (60:40, v/v). The flow rate and temperature of the column were set as 0.8 mL/min and 25 °C, respectively. The instrument detection limits of TX-45, IOPA, CIP and BPA for an injection volume of 100 µL were calculated as 28, 78, 3.3 and 3.0 µg/L, respectively. Anion analysis was conducted using a Dionex ICS-1500 ion chromatography unit equipped with a conductivity detector, a Dionex IonPac AG14A (4 × 50 mm) guard column and a Dionex IonPac AS14A (4 × 250 mm) analytical column. All other analyses for conventional parameters were performed as defined in *Standard Methods* (APHA 2005).

Acute toxicity and genotoxic activity measurements

Before conducting the toxicity tests, residual PS in the test samples was removed with sodium thiosulfate (Olmez-Hanci *et al.* 2014). In order to eliminate their interference with the toxicity and genotoxicity test results, Al^{3+} residuals in the reaction solutions were removed in the form of $Al(OH)_3$ flocs by pH adjustment to 6–7 using 1 N NaOH, precipitation and membrane filtration steps prior to acute toxicity/genotoxicity analysis.

Acute toxicity

The toxicity towards the photobacterium *V. fischeri* was measured with a BioTox™ test kit (Aboatox Oy, Finland) based on the percent relative luminescence inhibition after an incubation period of 15 minutes in accordance with the ISO 11348-3 (2008) protocol. The acute toxicity towards the freshwater microalga *P. subcapitata* was determined using Algaltoxkit F™ (MicroBioTests, Inc., Ghent, Belgium) microbiotests according to the procedure described in ISO 8692 (2012). Percent relative growth inhibition rates were calculated after an incubation period of 72 h on the basis of a toxicant-free control. The application of these standard methods for the assessment of toxicity on *V. fischeri* and *P. subcapitata* has been described in more detail elsewhere (Olmez-Hanci *et al.* 2014). Acute toxicity results were reported as percent relative inhibition with respect to a control during MPs treatment in DW and RSW samples. A positive control sample with potassium dichromate was also included for each test and all bioassays were run in triplicate.

Genotoxicity

Test systems to determine the genotoxicity or mutagenicity can be grouped by the biological system employed and their genetic endpoint detected. Bioassays with prokaryotes (i.e., *Salmonella*) enable the detection of agents that induce gene mutation and primary DNA damages. Bioassays using *Salmonella typhimurium* strain TA1535/pSK1002 have been developed and standardized (ISO 13829 2000) for the determination of genotoxicity. Additionally, the inhibition of bacterial growth could be used as a parameter to measure cytotoxic potential of the samples (Masood & Malik 2013). In the present study, the umuC test (in triplicate) was carried out according to the procedure described by ISO 13829 (2000). The UMU-Chromo Test™ test kit was supplied by EBPI Environmental Bio-Detection Products Inc. (Canada). According to the ISO 13829 (2000) protocol,

genotoxicity is measured by quantifying the induction ratio as the ratio of β-galactosidase activity of samples and controls in relation to the growth inhibition of the bacteria *Salmonella typhimurium* TA1535 (pSK1002). In the present study, several dilutions (namely 1:1.5, 1:3.0, 1:6 and 1:12) of the original and treated MP samples together with positive, negative and solvent controls were tested in accordance with the test protocol. For each sample dilution, the growth factor G, the β-galactosidase activity U_S and induction ratio I_R were calculated as given below:

$$G = \left[\frac{A_{600,S} - A_{600,B}}{A_{600,NC} - A_{600,B}}\right] \tag{6}$$

$$U_S = \left[\frac{A_{420,S} - A_{420,B}}{A_{600,S} - A_{600,B}}\right] \tag{7}$$

$$I_R = \left(\frac{1}{G}\right) \times \left[\frac{A_{420,S} - A_{420,B}}{A_{420,NC} - A_{420,B}}\right] \tag{8}$$

where $A_{600,S}$ and $A_{420,S}$ are the absorbance of sample S at 600 nm and 420 nm; $A_{600,B}$ and $A_{420,B}$ are the absorbance of the blank B at 600 nm and 420 nm; $A_{600,NC}$ and $A_{420,NC}$ are the absorbance of the negative control (NC) at 600 nm and 420 nm. Wavelengths of 600 nm and 420 nm were used to measure bacterial growth and quantify β-galactosidase activity, respectively. The optical density was measured using a Bio Rad Benchmark Plus microplate spectrophotometer. The whole test was considered valid if G > 0.5 and the positive controls reach an I_R value >2. Calculation of G allowed identification of toxic growth inhibitory effects, and G < 0.5, representing >50% inhibition of the biomass growth, was considered to be indicative of samples being cytotoxic. An I_R > 1.5 was taken as the threshold at which the sample was considered as genotoxic (ISO 13829 2000).

RESULTS AND DISCUSSION

Treatability of MPs with ZVA and ZVA-activated PS

In order to assess the oxidation capacity of the PS/ZVA treatment system for TX-45, IOPA, CIP and BPA degradation, some preliminary experiments were conducted in DW (MPs = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM or 0.50 mM; pH = 3; t = 120 min). Additionally, the degradation of MPs with mere PS oxidation (in the absence of ZVA) and ZVA/

H^+/O_2 treatment (in the absence of PS) was also examined. MPs abatement profiles as a function of treatment time for mere PS oxidation and the $ZVA/O_2/H^+$ system are presented in Supporting Information Figures S1 and S2, respectively (available with the online version of this paper). Table 1 also summarizes percent overall MPs removal efficiencies obtained from these preliminary experiments. As can be seen from Figures S1 and S2 as well as Table 1, TX-45, IOPA and BPA abatements were poor for mere PS oxidation and for $ZVA/H^+/O_2$ treatment as well. These results indicated that the $ZVA/H^+/O_2$ treatment system as well as mere PS oxidation were not effective for the removal of TX-45, IOPA and BPA. On the other hand, high removal efficiency was achieved for CIP (58%) at the end of 120-minute $ZVA/O_2/H^+$ treatment. As aforementioned *in situ* generated HP in the aqueous suspension of ZVA serves as a precursor of the $HO^{\cdot}$, which is primarily responsible for the effective CIP oxidation in the $ZVA/H^+/O_2$ system (Bokare & Choi 2009).

As expected, PS activation with ZVA greatly enhanced the degradation rates of MPs as compared to mere PS oxidation and $ZVA/H^+/O_2$ treatment. External addition of 0.25 mM PS to the reaction medium increased the TX-45 and IOPA removal efficiencies to 86% and 75%, respectively, at the end of 120-minute PS/ZVA treatment. Nonetheless CIP and BPA were completely degraded after 120-minute and 40-minute treatment with the PS/ZVA (0.25 mM) process. As can be seen from Table 1 increasing the initial PS concentration improved the TX-45 (from 86% to 100%) and IOPA (from 75% to 95%) removals. In contrast, CIP removal efficiencies decreased with increasing the initial PS concentration from 0.25 mM (100%) to 0.50 mM (69%). Correspondingly, the time required for complete removal of BPA increased with increasing the initial

PS concentration. These observations could be attributed to the well-known $SO_4^{\cdot-}$ scavenging effect of excessive PS (Arslan-Alaton et al. 2017a).

Effect of the water matrix

In order to assess the process performance of the PS/ZVA treatment process for MPs removal in RSW samples, a set of degradation experiments were conducted under the following optimized reaction conditions: MPs = 2 mg/L; ZVA = 1 g/L; pH = 3; PS = 0.25 mM (for CIP and BPA) and 0.50 mM (for TX-45 and IOPA). Figure 1(a) and 1(b) delineates time-dependent changes in TX-45, IOPA, CIP and BPA concentrations during PS/ZVA treatment in DW and RSW, respectively. In the natural water sample, a dramatic decrease in TX-45, IOPA, CIP and BPA removal rates was observed owing to the presence of organic and

Figure 1 | Time-dependent changes in TX-45, IOPA, CIP and BPA concentrations during PS/ZVA treatment in DW (a) and RSW (b). Treatment conditions: MP = 2 mg/L; DOC_{RSW} = 7.2 mg/L; ZVA = 1 g/L; PS = 0.25 mM for CIP and BPA; PS = 0.50 mM for TX-45 and IOPA; pH = 3; T = 25 °C. Error bars are standard deviations of triplicate measurements.

Table 1 | Comparison of overall percent MP removal efficiencies obtained after 120-minute PS oxidation as well as $ZVA/O_2/H^+$ and PS/ZVA treatments in DW

| Treatment system	MP removals (%)			
	TX-45	IOPA	CIP	BPA
PS (0.50 mM PS)	20	10	10[a]	10
$ZVA/O_2/H^+$	22	8	58	18
PS/ZVA (0.25 mM PS)	86	75	100	100[b]
PS/ZVA (0.50 mM PS)	100[c]	95	69	100[d]

Treatment conditions: MP = 2 mg/L; ZVA = 1 g/L; pH = 3; T = 25 °C.
[a]PS = 0.25 mM.
[b]40 min.
[c]90 min.
[d]90 min.

inorganic competitors for $SO_4^{\bullet-}$. The corresponding TX-45, IOPA, CIP and BPA removal efficiencies in RSW were 59%, 29%, 73% and 46%, respectively. These attained results are consistent with the presence of organic (i.e., natural organic matter) and inorganic (i.e., nitrate, chloride, phosphate) compounds present in the RSW sample capable of scavenging $SO_4^{\bullet-}$. It is expected that the presence of water components inhibits oxidation of MPs. Similar results were also obtained by Baeza & Knappe (2011) who suggested that the presence of radical scavengers in lake water and wastewater treatment plant effluent matrices was the dominant factor that decreased biochemically active compound (sulfamethoxazole, sulfamethazine, sulfadiazine, trimethoprim, BPA and diclofenac) transformation rates. Dimitroula et al. (2012) also observed a decrease in the oxidation rate of several MPs of one order of magnitude when comparing the reaction in pure water with a wastewater matrix. Moreover, advanced oxidation of organic pollutants in real water matrices might lead to the formation of different intermediates types than in pure water that could lower overall process efficiency, since these species could be less reactive with the $SO_4^{\bullet-}$ or $HO^{\bullet}$ (Kiwi et al. 2000).

Changes in acute toxicity

Considering that the degradation and toxicity pattern of MPs will be affected by the characteristics of the natural water sample, changes in acute toxicity and genotoxicity were examined during PS/ZVA treatment of MPs in DW and RSW. Percent relative inhibition values before ($t = 0$) and during PS/ZVA treatment of MPs in DW and RSW samples are presented for V. fischeri and P. subcapitata in Figures 2 and 3, respectively. Toxicity analysis of the original and PS/ZVA-treated MP samples demonstrated different patterns for the studied test organisms (V. fischeri and P. subcapitata) as well as the water matrix. The percent relative inhibitions of the original (untreated) TX-45, IOPA, CIP and BPA samples in DW towards V. fischeri were in decreasing order of CIP (51%) > BPA (40%) > TX-45 (15%) > IOPA (1%). On the other hand, in the natural water sample bearing MPs, the decreasing order of relative inhibitory effects were obtained as CIP (42%) > IOPA (27%) > BPA (12%) > TX-45 (6%). From the above findings it might be inferred that the V. fischeri toxicities in the DW samples were suppressed as compared with the RSW, except for IOPA. The original IOPA in the DW sample was practically non-toxic towards V. fischeri, whereas an inhibitory effect of 27% was observed for RSW sample. CIP appeared to be the most toxic MP towards V. fischeri for both water matrices.
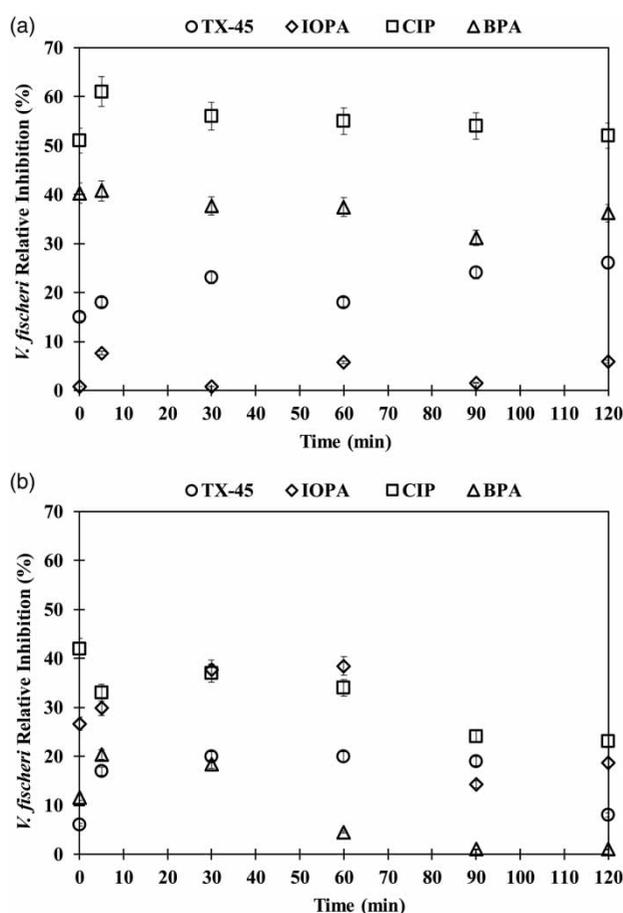
Figure 2 | V. fischeri acute toxicity evolution based on percent relative inhibition rates observed during PS/ZVA treatments in DW (a) and RSW (b). Treatment conditions as in Figure 1. Error bars are standard deviations of triplicate measurements.

The obtained findings indicate that the toxicity results depend on complex interaction between the pollutant type (its physicochemical properties), water matrix and test organism.

As can be seen from Figure 2(a) and 2(b), the inhibitory effect of aqueous MP solutions towards V. fischeri fluctuated in the case of PS/ZVA treatment both in DW and RSW, most probably due to the formation and subsequent oxidation of MP degradation products. The V. fischeri toxicities of CIP and BPA remained nearly constant throughout the PS/ZVA treatment in DW, determined as 52% and 36%, respectively, after 120-minute treatment. PS/ZVA-treated TX-45 in DW resulted in a slightly higher toxicity towards V. fischeri (26%) than in RSW. As is reported in the related literature, the step-wise loss of the side chain ethoxylate units of alkylphenol ethoxylates typically results in more hydrophobic and hence more toxic intermediates (Hill & Jans 2003). In the case of IOPA, 120-minute
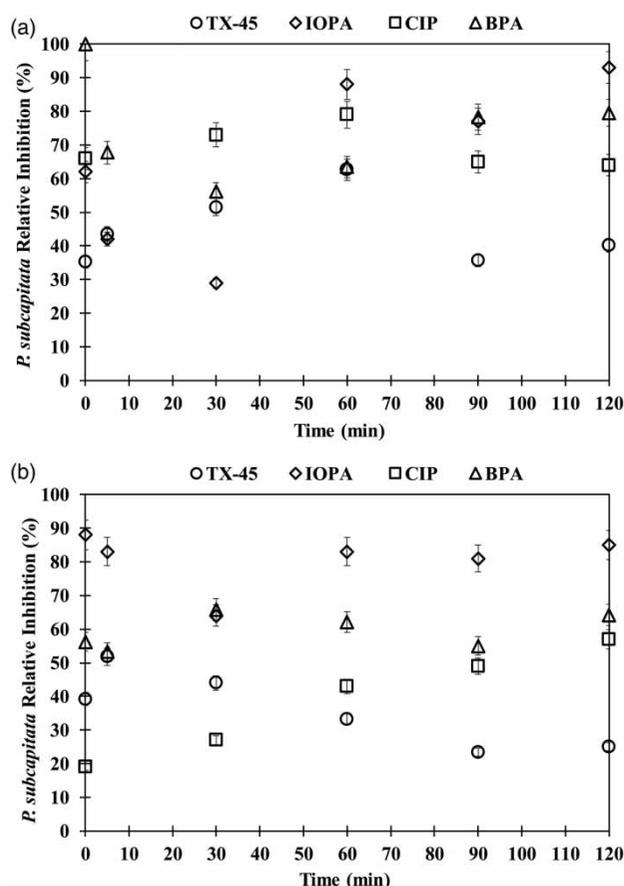
Figure 3 │ *P. subcapitata* acute toxicity evolution based on percent relative inhibition rates observed during PS/ZVA treatments in DW (a) and RSW (b). Treatment conditions as in Figure 1. Error bars are standard deviations of triplicate measurements.

PS/ZVA treatment in DW did not result in toxic degradation products towards *V. fischeri*. The original *V. fischeri* toxicities of CIP, IOPA, BPA and TX-45 were 42%, 27%, 12% and 6%, respectively. It is apparent from Figure 2(b) that, after 120-minute PS/ZVA treatment, TX-45 (8%) and BPA (1%) were found to be practically non-toxic, whereas 19% and 23% relative inhibitions were still evidenced for IOPA and CIP, respectively.

According to the *P. subcapitata* growth inhibition test results given in Figure 3(a) and 3(b), the untreated BPA (100%), CIP (66%) and IOPA (62%) in DW samples were highly toxic. On the other hand, untreated TX-45 (35%) was not as toxic as BPA, CIP and IOPA. Similar to *V. fischeri* toxicities, the inhibitory effect of the original BPA and CIP samples was suppressed in RSW, measured as 56% for BPA and 19% for CIP. It is interesting to point out that the *P. subcapitata* toxicity of IOPA in RSW sample increased to 88%, whereas TX-45 toxicity in RSW (39%) was close to the value being observed in DW (35%).
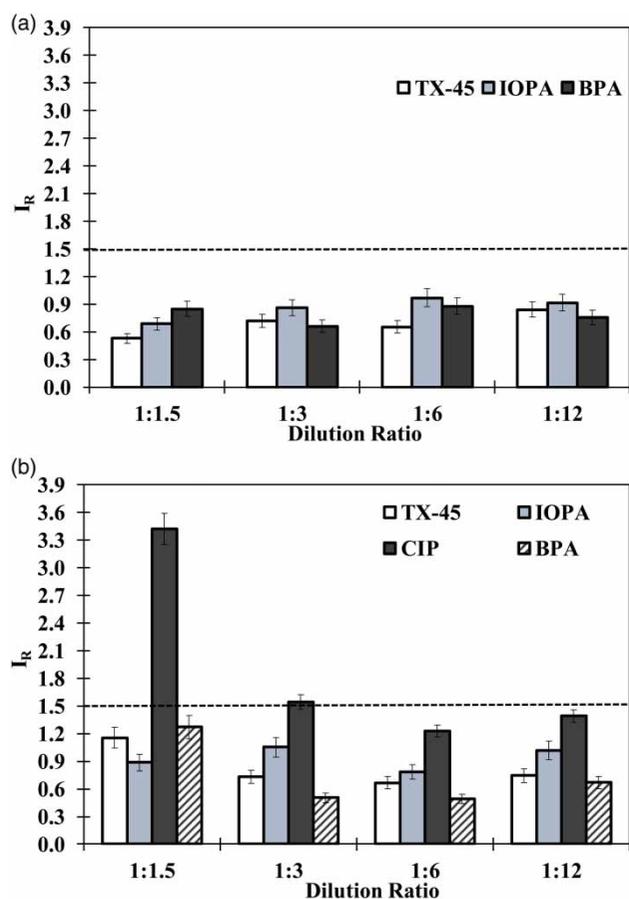
The increasing and decreasing trend of toxicity towards *P. subcapitata* was also evidenced during PS/ZVA treatment of the investigated MPs in DW and RSW samples. As can be seen from Figure 3(a) the acute inhibitory effect showed no significant change within 120-minute PS/ZVA treatment of TX-45 (from 35% to 40%) and CIP (from 66% to 64%) in DW. On the other hand, the untreated IOPA in DW showed an increase in its inhibitory effect from 62% to 93% after 120-minute treatment, most possibly due to the presence of some degradation products being more toxic than the original IOPA solution (Arslan-Alaton et al. 2017b). In the case of BPA in DW, the relative inhibition of 100% decreased to 80% after 120-minute treatment time, which was still very high.

In general, the bioassay performed with *P. subcapitata* in DW appeared to be more sensitive to the investigated MPs degradation products among the selected bioassays. *P. subcapitata* toxicity patterns differed appreciably for RSW samples; during PS/ZVA treatment of BPA, percent relative inhibition increased from 56% to 64% after 120 minutes, which was not practically different from that of the untreated BPA. In the case of IOPA, the relative inhibition fluctuated during the treatment and reached a value (85%) which was at the same level of the untreated IOPA (88%). On the other hand, TX-45 degradation products exhibited a low toxic effect towards *P. subcapitata* after 120 minutes (around 25%). For PS/ZVA treatment of CIP in RSW, *P. subcapitata* toxicity of CIP increased from 19% to 57% after 120 minutes, attesting to the fact that degradation products were more toxic towards *P. subcapitata* than the original CIP.

It should be pointed out here that the removal efficiencies of the investigated MPs and aquatic toxicity patterns being observed before, during and after treatment with PS/ZVA process in DW and RSW matrices were quite different from each other. Furthermore, the sensitivity of microorganism used in toxicity tests towards MPs and their degradation products was found to be different. Consequently, based on the data obtained in this study, it is advisable to adopt different specific toxicity tests to refine the identification of toxic/inhibitory effects linked to the presence of MPs in different water matrices.

Changes in genotoxic activity

Figure 4 presents the I_R values obtained for the untreated and PS/ZVA-treated TX-45, IOPA, CIP and BPA in DW. The calculated G values demonstrated that neither the original nor the treated products of TX-45, IOPA and BPA were cytotoxic. As aforementioned, in the ISO 13829 test

Figure 4 | Evolution of genotoxic effects of the untreated (a) and PS/ZVA-treated (b) TX-45, IOPA, CIP and BPA samples in DW. Treatment conditions as in Figure 1. Error bars are standard deviations of triplicate measurements.

TA102 (Ames test) in the absence of an exogenous metabolizing system. In a recent study, CIP and samples of photolyzed aqueous CIP exerted heritable genotoxic effects in the HepG2 cell line (Garcia-Käufer et al. 2012). In this study, an aqueous CIP solution at a concentration of 0.4 mg/L (1.2 μmol/L) was found to meet the lowest observed effect level, as was assessed previously by dose-response experiments. It was also shown that the photolytic decomposition products of CIP and other fluoroquinolones are more genotoxic than the drug itself (Sanchez et al. 2005; Pereira et al. 2007). As has been emphasized in Garcia-Käufer et al. (2012), for the induction of heritable mutations or cancer in humans involved with exposure to CIP or its transformation products, more detailed studies, especially those applying in vivo toxicity tests, will be necessary.

CONCLUSIONS AND RECOMMENDATIONS

In the present study, activation of PS with nanoscale ZVA (1 g/L) particles was investigated in DW and RSW samples for the treatment of 2 mg/L TX-45, IOPA, CIP and BPA at pH 3 for 120 minutes. Changes in acute toxicity and genotoxicity patterns during PS/ZVA treatment of selected MPs were particularly studied. The major findings of this work are summarized below:

- Mere PS oxidation resulted in MP removals in the range of 10–22%.
- ZVA/O_2/H^+ treatment led to inefficient degradation of TX-45 (22%), IOPA (8%), CIP (58%) and BPA (18%) at the studied treatment conditions: MP = 2 mg/L; ZVA = 1 g/L; pH = 3; T = 25 °C; t = 120 min.
- On the other hand, PS activation with nanoscale ZVA greatly enhanced the MP removals, resulting in complete CIP (in 120 minutes) and BPA (in 40 minutes) degradation, whereas 86% and 75% removal efficiencies were realized for TX-45 and IOPA, respectively, with PS/ZVA treatment (MP = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM; pH = 3; T = 25 °C; t = 120 min).
- The decreasing order of MP removal efficiencies in DW at the optimized treatment conditions (MP = 2 mg/L; ZVA = 1 g/L; PS = 0.25 mM for CIP and BPA; PS = 0.50 mM for TX-45 and IOPA; pH = 3; T = 25 °C) were; BPA (100% in 40 minutes) > TX-45 (100% in 90 minutes) > CIP (100% in 120 minutes) > IOPA (95% in 120 minutes).
- The environmental characteristics of the water matrix (its inorganic and organic matter content) represented a

protocol, a growth factor (G) of less than 0.5 (representing 50% inhibition of biomass growth) is considered to be indicative of cytotoxic samples, and as indicated in the validity criteria of the above test protocol, the results cannot be evaluated if G < 0.5. In the present study, only CIP was found to be cytotoxic (G = 0.23) at the studied dilution ratios and due to G < 0.5 the I_R could not be calculated. Both untreated (Figure 4(a)) and PS/ZVA-treated (Figure 4(b)) TX-45, IOPA and BPA samples had no genotoxic activity according to the umuC test results. However, PS/ZVA treatment of CIP in DW caused the formation of genotoxic degradation products after 120 minutes. The induction ratios of 1.0:1.5 and 1.0:3.0 dilutions were calculated as 3.42 and 1.54, respectively, for the PS/ZVA-treated CIP samples. As indicated in former related studies (Mersch-Sundermann et al. 1994; Kümmerer et al. 2000), topoisomerases-II inhibitors like CIP and ofloxacin are very strong genotoxicants for Escherichia coli PQ37 (SOS Chromo test) and mutagens for Salmonella typhimurium

drawback for MPs removal. The efficiency of the PS/ZVA treatment system in RSW after 120 minutes followed the decreasing order of CIP (73%) > TX-45 (59%) > BPA (46%) > IOPA (29%).

- The percent relative inhibitions of the original TX-45, IOPA, CIP and BPA samples in DW were in order of CIP (51%) > BPA (40%) > TX-45 (15%) > IOPA (1%) towards *V. fischeri*, whereas the decreasing order was found as BPA (100%) > CIP (66%) > IOPA (62%) > TX-45 (35%) towards *P. subcapitata*.

- Acute toxicities of the original BPA and CIP samples were suppressed in the natural water samples. In the case of original IOPA, *V. fischeri* and *P. subcapitata* toxicities in RSW increased to 27% and 88%, respectively, compared to DW. *P. subcapitata* toxicity of TX-45 in DW and RSW samples were found to be at the same level (35–39%) whereas a lower *V. fischeri* toxicity was evidenced in RSW (6%) sample than in DW (15%).

- The *V. fischeri* and *P. subcapitata* toxicities fluctuated during PS/ZVA treatment both in DW and RSW samples and resulted in different relative inhibition values at the end of 120-minute treatment.

- According to the UMU-Chromo test results, the original and PS/ZVA-treated TX-45, IOPA and BPA samples exhibit neither cytotoxic nor genotoxic effects. Only CIP was found to be cytotoxic at an initial concentration of 2 mg/L and PS/ZVA-treated CIP resulted in the formation of genotoxic degradation products.

ACKNOWLEDGEMENTS

REFERENCES

Anipsitakis, G. P. & Dionysiou, D. D. 2004 Transition metal/UV-based advanced oxidation technologies for water decontamination. *Applied Catalysis B – Environmental* **54**, 155–163.

Antoniou, M. G., de la Cruz, A. A. & Dionysiou, D. D. 2010 Degradation of microcystin-LR using sulfate radicals generated through photolysis, thermolysis and e-transfer mechanisms. *Applied Catalysis B – Environmental* **96**, 290–298.

APHA 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Public Health Association/

American Water Works Association/Water Environment Federation, Washington, DC, USA.

Arslan-Alaton, I., Olmez-Hanci, T., Khoei, S. & Fakhri, H. 2017a Oxidative degradation of Triton X-45 using zero valent aluminum in the presence of hydrogen peroxide, persulfate and peroxymonosulfate. *Catalysis Today* **280**, 199–207.

Arslan-Alaton, I., Olmez-Hanci, T., Korkmaz, G. & Sahin, C. 2017b Removal of iopamidol, an iodinated X-ray contrast media, by zero-valent aluminum-activated H_2O_2 and $S_2O_8^{2-}$ oxidation. *Chemical Engineering Journal* **318**, 64–75.

Baeza, C. & Knappe, D. R. U. 2011 Transformation kinetics of biochemically active compounds in low-pressure UV photolysis and UV/H_2O_2 advanced oxidation processes. *Water Research* **45**, 4531–4543.

Bokare, A. D. & Choi, W. 2009 Zero-valent aluminum for oxidative degradation of aqueous organic pollutants. *Environmental Science and Technology* **43**, 7130–7135.

Cheng, Z., Fu, F., Pang, Y., Tang, B. & Lu, J. 2015 Removal of phenol by acid-washed zero-valent aluminium in the presence of H_2O_2. *Chemical Engineering Journal* **260**, 284–290.

Dimitroula, H., Daskalaki, V. M., Frontistis, Z., Kondarides, D. I., Panagiotopoulou, P., Xekoukoulotakis, N. P. & Mantzavinos, D. 2012 Solar photocatalysis for the abatement of emerging micro-contaminants in wastewater: synthesis, characterization and testing of various TiO_2 samples. *Applied Catalysis B – Environmental* **117–118**, 283–291.

Escher, B., Allinson, M., Altenburger, R., Bain, P. A., Balaguer, P., Busch, W., Crago, J., Denslow, N. D., Dopp, E., Hilscherova, K., Humpage, A. R., Kumar, A., Grimaldi, M., Jayasinghe, B. S., Jarosova, B., Jia, A., Makarov, S., Maruya, K. A., Medvedev, A., Mehinto, A. C., Mendez, J. E., Poulsen, A., Prochazka, E., Richard, J., Schifferli, A., Schlenk, D., Scholz, S., Shiraish, F., Snyder, S., Su, G. Y., Tang, J. Y. M., van der Burg, B., van der Linden, S. C., Werner, I., Westerheide, S. D., Wong, C. K. C., Yang, M., Yeung, B. H. Y., Zhang, X. W. & Leusch, F. D. L. 2014 Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environmental Science and Technology* **48**, 1940–1956.

Fent, K., Weston, A. A. & Carminada, D. 2006 Ecotoxicology of human pharmaceuticals. *Aquatic Toxicology* **76**, 122–159.

Garcia-Käufer, M., Haddad, T., Bergheim, M., Gminski, R., Gupta, P., Mathur, N., Kümmerer, K. & Mersch-Sundermann, V. 2012 Genotoxic effect of ciprofloxacin during photolytic decomposition monitored by the in vitro micronucleus test (MNvit) in HepG2 cells. *Environmental Science Pollution Research* **19**, 1719–1727.

Gavrilescu, M., Demnerov, K., Aamand, J., Agathos, S. & Fava, F. 2015 Emerging pollutants in the environment: present and future challenges in biomonitoring, ecological risks and bioremediation. *New Biotechnology* **32**, 147–156.

Girit, B., Dursun, D., Olmez-Hanci, T. & Arslan-Alaton, I. 2015 Treatment of aqueous bisphenol A using nano-sized zero-valent iron in the presence of hydrogen peroxide and persulfate oxidants. *Water Science and Technology* **71**, 1859–1868.

Hamer, B., Bihari, N., Reifferscheid, G., Zahn, R. K., Muller, W. E. & Batel, R. 2000 Evaluation of the SOS/umu-test

post-treatment assay for the detection of genotoxic activities of pure compounds and complex environmental mixtures. *Mutation Research* **23**, 161–171.

Hill, R. L. & Jans, D. M. 2003 Developmental estrogenic exposure in zebrafish (*Danio rerio*): I. Effects on sex ratio and breeding success. *Aquatic Toxicology* **63**, 417–429.

Huang, K. C., Zhao, Z. Q., Hoag, G. E., Dahmani, A. & Block, P. A. 2005 Degradation of volatile organic compounds with thermally activated persulfate oxidation. *Chemosphere* **61**, 551–560.

ISO 11348-3 2008 *Water Quality – Determination of the Inhibitory Effect of Water Samples on the Light Emission of* Vibrio fischeri. International Organisation for Standardization, Geneva.

ISO 13829 2000 *Water Quality – Determination of the Genotoxicity of Water and Waste Water Using the Umu-Test.* International Organisation for Standardization, Geneva.

ISO 8692 2012 *Water Quality – Freshwater Algal Growth Inhibition Test with Unicellular Green Algae.* International Organisation for Standardization, Geneva.

Kiwi, J., Lopez, A. & Nadtochenko, V. 2000 Mechanism and kinetics of the ·OH-radical intervention during Fenton oxidation in the presence of a significant amount of radical scavenger (Cl⁻). *Environmental Science and Technology* **34**, 2162–2168.

Kümmerer, K., Al-Ahmad, A. & Mersch-Sundermann, V. 2000 Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. *Chemosphere* **40**, 701–710.

Luo, Y., Guo, W., Ngo, H. H., Nghiem, L. D., Hai, F. I., Zhang, J., Liang, S. & Wang, X. C. 2014 A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Science of the Total Environment* **473–474**, 619–641.

Masood, F. & Malik, A. 2013 Mutagenicity and genotoxicity assessment of industrial wastewaters. *Environmental Science and Pollution Research* **20**, 7386–7397.

Mersch-Sundermann, V., Schneider, U., Klopman, G. & Rosenkranz, H. S. 1994 SOS-induction in *E. coli* and *Salmonella* mutagenicity: a comparison using 330 compounds. *Mutagenesis* **9**, 205–224.

Olmez-Hanci, T., Arslan-Alaton, I. & Dursun, D. 2014 Investigation of the toxicity of common oxidants used in advanced oxidation processes and their quenching agents. *Journal of Hazardous Materials* **278**, 330–335.

Papa, M., Ceretti, E., Viola, G. C. V., Feretti, D., Zerbini, I., Mazzoleni, G., Steimberg, N., Pedrazzani, R. & Bertanza, G. 2016 The assessment of WWTP performance: towards a jigsaw puzzle evaluation? *Chemosphere* **145**, 291–300.

Pasquini, L., Munoz, J.-F., Pons, M.-N., Yvon, J., Dauchy, X., France, X., Le, N. D., France-Lanord, C. & Görner, T. 2014 Occurrence of eight household micropollutants in urban wastewater and their fate in a wastewater treatment plant. Statistical evaluation. *Science of the Total Environment* **481**, 459–468.

Pereira, V. J., Linden, K. G. & Weinberg, H. S. 2007 Evaluation of UV for photolytic and oxidative degradation of pharmaceutical compounds in water. *Water Research* **41**, 4413–4423.

Rizzo, L. 2011 Bioassays as a tool for evaluating advanced oxidation processes in water and wastewater treatment. *Water Research* **45**, 4311–4340.

Sanchez, G., Hidalgo, M. E., Vivanco, J. M. & Escobar, J. 2005 Induced and photoinduced DNA damage by quinolones: ciprofloxacin, ofloxacin and nalidixic acid determined by comet assay. *Journal of Photochemistry and Photobiology* **81**, 819–822.

Žegura, B., Heath, E., Černoša, A. & Filipič, M. 2009 Combination of *in vitro* bioassays for the determination of cytotoxic and genotoxic potential of wastewater, surface water and drinking water samples. *Chemosphere* **75**, 1453–1460.

Zhang, H., Cao, B., Liu, W., Lin, K. & Feng, J. 2012 Oxidative removal of acetaminophen using zero valent aluminum-acid system: efficacy, influencing factors, and reaction mechanism. *Journal of Environmental Science* **24**, 314–319.